Published in final edited form as:

Brain Res. 2019 July 15; 1715: 106–114. doi:10.1016/j.brainres.2019.03.011.

Effects of early-life FGF2 on ultrasonic vocalizations (USVs) and the mu-opioid receptor in male Sprague-Dawley rats selectively-bred for differences in their response to novelty

Cortney A. Turner¹, Megan H. Hagenauer¹, Elyse L. Aurbach¹, Pamela M. Maras¹, Chelsea L. Fournier¹, Peter Blandino Jr.¹, Rikav B. Chauhan¹, Jaak Panksepp^{2,#}, Stanley J. Watson Jr.^{1,3}, and Huda Akil^{1,3}

¹Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI, USA

²Department of Integrative Physiology & Neuroscience, Washington State University, Pullman, WA, USA

³Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA

Abstract

In previous studies, early-life fibroblast growth factor-2 (FGF2) administration conferred resilience to developing anxiety-like behavior in vulnerable animals in adulthood. To follow up on this work, we administered FGF2 the day after birth to animals that differ in emotional behavior and further explored its long-term effects on affective behavior and circuitry. Selectively-bred “high responder” rats (bHRs) exhibit low levels of anxiety-like and depression-like behavior, whereas selectively-bred “low responders” (bLRs) display high levels of anxiety-like and depression-like behavior. We found that early-life administration of FGF2 decreased negative affect in bLRs during the early post-natal period, as indexed by 40 kHz ultrasonic vocalizations (USVs) in response to a brief maternal separation on PND11. FGF2 also increased positive affect during the juvenile period, as measured by 50 kHz USVs in response to heterospecific hand play (“tickling”) after weaning. In general, we found that bHRs produced more 50 kHz USVs than bLRs. In adulthood, we measured opioid ligand and receptor expression in brain regions implicated in USV production and affect regulation by mRNA *in situ* hybridization. Within multiple affective brain regions, bHRs had greater expression of the mu opioid receptor than bLRs. FGF2 increased mu opioid expression in bLRs. The bLRs had more kappa and less delta receptor expression than bHRs, and FGF2 increased prodynorphin in bLRs. Our results provide support for further investigations into the role of growth factors and endogenous opioids in the treatment of disorders characterized by altered affect, such as anxiety and depression.

Correspondence: Dr. Cortney Turner, Molecular & Behavioral Neuroscience Institute, University of Michigan, 205 Zina Pitcher Pl, Ann Arbor, MI, 48109, USA, cturner@umich.edu, Phone: (734) 936-2034, Fax: (734) 647-4130.

[#]Note: Jaak Panksepp – deceased

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

ultrasonic vocalizations; fibroblast growth factor-2; opioid; separation distress; affect; development

1. Introduction

The underlying neurobiology of positive and negative affect has profound implications for many neuropsychiatric disorders, including Major Depressive Disorder and anxiety disorders, but the developmental emergence of these emotional states remains poorly understood. In our laboratory, we model the development of affective temperament using rodents that have been selectively-bred to capture two extremes of emotional reactivity. The selectively-bred high responder rats (bHRs) are highly exploratory in a novel environment and prone to impulsive, aggressive, and reward-seeking behavior, whereas the selectively-bred low-responders (bLRs) are highly inhibited in a novel environment, extremely anxious and prone to developing depressive-like behavior (Clinton et al., 2007; Perez et al., 2009; Stead et al., 2006; Turner et al., 2011).

One potential mediator of affective development is growth factors. Insulin growth factor-1 and brain-derived neurotrophic factor are known for their antidepressant or hedonic effects in adult animals (Burgdorf et al. 2017; Burgdorf et al. 2010; Hoshaw et al. 2005; Shirayama et al. 2002). Similarly, fibroblast growth factor-2 (FGF2) acts as an antidepressant and anxiolytic in adult animals (Chaudhury et al. 2014; Elsayed et al. 2012; Perez et al. 2009; Turner et al. 2008). However, early-life treatment with only one growth factor, FGF2, has been shown to decrease anxiety-like behavior in early life and adulthood (Litvin et al. 2016; Prater et al. 2017; Turner et al. 2011). These early-life FGF2 administration effects are particularly robust in the selectively-bred bLRs that also exhibit elevated anxiety-like behavior (Turner et al. 2011). Interestingly, bLRs and bHRs also exhibit basal differences in FGF2 gene expression in adulthood, implying that FGF2 may play an important role in the development of emotional regulation (Eren-Kocak et al. 2011; Perez et al. 2009). The current work extends our understanding of early-life FGF2 effects in the bHR/bLR model by examining positive and negative affective responses during development as well as relevant neural circuitry regulating affect in the adult brain.

To date, one of the clearest measures of positive and negative affect in laboratory rodents are ultrasonic vocalizations (USVs) (Wright et al., 2010). These USVs can be easily elicited under laboratory conditions in young animals. During early development, a brief five-minute maternal separation on postnatal day 11 (PND11) vigorously elicits 40 kHz separation distress USVs (Litvin et al. 2016). Later in development and adulthood, negatively-valenced calls are emitted in the 22 kHz range, whereas positively-valenced calls are emitted at higher frequencies (“50 kHz calls”) (Burgdorf et al. 2008). In juveniles, the 50 kHz USVs are reliably elicited by rough-and-tumble play (Knutson et al. 2002), which can be simulated in the laboratory using heterospecific hand play (i.e. “tickling”) (Burgdorf et al. 2011). Importantly, this USV response of animals to tickling has been previously shown to be modulated by growth factors (Burgdorf et al. 2011).

The neural circuitry responsible for the regulation of negative and positive affect overlaps extensively with the circuitry responsible for the generation of emotional USVs. This includes many regions with dense opioid receptor binding, such as the ventral septum, preoptic area (POA), bed nucleus of the stria terminalis (BNST), dorsomedial thalamus (dmThal), nucleus accumbens (NAcc), and periaqueductal gray (PAG) (Burgdorf et al. 2001a; Burgdorf et al. 2007; Panksepp et al. 1980; Panksepp 1988), as well as the cingulate cortex (ACC) (Panksepp 2003; 2005; 1988). Endogenous opioids have been found to play a critical role in both separation distress and rewarding social interactions (Burgdorf and Panksepp 2001; Machin AJ 2011; Panksepp et al. 1980; Panksepp et al. 1997), and pharmacological agents selectively targeting the opioid system can modulate positive affect (Berrocoso and Mico 2009; Lutz and Kieffer 2013; Torregrossa et al. 2005; Torregrossa et al. 2006). Opiate agonists and antagonists have been shown to modulate positive and negative USVs (Burgdorf et al., 2001; Knutson et al., 1999; Vivian and Miczek, 1993), including the USV response of animals to tickling (Burgdorf and Panksepp 2001).

We hypothesized that bHRs and bLRs would differ in their positive and negative affect early in life. Specifically, USV responses to heterospecific hand play and maternal separation would differ, and the bLRs administered FGF2 early in life would resemble bHRs during behavioral tests. We also hypothesized that bHRs and bLRs would exhibit basal differences in opioid system gene expression within the circuitry that regulates both affect and USV calls. Finally, we hypothesized that early-life treatment with FGF2 might make opioid system gene expression in the bLRs resemble that of the bHRs.

2. Results

2.1 Early-life FGF2 decreased maternal separation-induced USVs in bLRs on PND11

We first sought to determine whether early-life FGF2 treatment (PND1) would reduce separation distress vocalizations (40 kHz USVs) on PND11 in a selectively-bred model of response to a novel environment. During separation from the dam, there was a significant interaction between the effects of phenotype and treatment on separation-induced USVs ($n=11-15$; $F(1,50)=7.49$, $p=0.009$). As shown in Fig. 1, when the pups were treated with vehicle, there was a nonsignificant trend for the highly-anxious bLR animals to exhibit more separation-induced USVs than bHR animals ($p=0.069$). FGF2 treatment decreased the number of USVs in bLR animals so that bLR-VEH showed more separation-induced USVs than bLR-FGF2 animals ($p<0.05$). There was, however, no main effect of FGF2 treatment ($n=11-15$; $F(1,50)=1.14$, $p=0.29$) or bHR/bLR phenotype ($n=11-15$; $F(1,50)=0.46$, $p=0.50$) on the number of separation-induced USVs. Therefore, the effects of early-life FGF2 were already evident in the early postnatal period and reduced maternal separation-induced USVs in bLRs.

2.2 Early-life FGF2 increased positive affect (50 kHz USVs) in juvenile bHRs and bLRs during exploration and tickling

Next, we examined whether early-life FGF2 was capable of increasing positive affect in a novel environment and in response to tickling as measured by 50 kHz USV production (Fig. 2a). During habituation to the testing arena (PND22), the effect of treatment depended on

the phenotype of the animal (*treatment*phenotype*: $F(1,40)=6.27$, $p=0.016$), with bHR-FGF2 animals showing more USVs than bHR-VEH ($p<0.001$), bLR-VEH ($p<0.001$) and bLR-FGF2 animals ($p<0.001$, see Fig. 2b). There was a main effect of phenotype on the production of positive 50 kHz USVs, with bHR animals exhibiting more USVs than the bLR animals ($F(1,40)=13.68$, $p=0.001$). There was also a main effect of treatment, with FGF2-treated animals exhibiting more USVs than vehicle animals ($F(1, 40)=13.23$, $p=0.001$). These results indicate that early-life FGF2 treatment can produce long-term increases in positive affect in response to exploring a novel environment, especially in individuals that already have a more positive response to novelty (bHRs).

During the first day of tickling, the effect of treatment depended on the phenotype of the animal (*treatment*phenotype*: $F(1,40)=8.56$, $p=0.006$). Here, bLR-VEH animals exhibited significantly fewer USVs than bLR-FGF2 animals ($p=0.017$) and bHR-VEH animals ($p=0.004$; see Fig. 2c). There was a main effect of phenotype on the production of positive 50 kHz USVs, with bHR animals exhibiting more USVs than the bLR animals ($F(1,40)=5.47$, $p=0.024$). The overall main effect of treatment was not significant ($F(1,40)=1.68$, $p=0.20$). These results suggest that early-life FGF2 treatment can produce long-term increases in positive affect in response to social stimulation in individuals that already have a less positive response to novelty (bLRs).

On the second day of tickling, there was no significant interaction ($F(1,39)=2.10$, $p=0.16$) and no main effect of treatment ($F(1,39)=1.03$, $p=0.32$). There was, however, a main effect of phenotype on the production of 50kHz USVs, with bHRs exhibiting more USVs than bLRs ($F(1,39)=19.31$, $p<0.001$; see Fig. 2d). On the third day of tickling, the interaction was not significant ($F(1,40)=0.23$, $p=0.632$). There was a main effect of phenotype, with the bHRs exhibiting more USVs than bLRs ($F(1,40)=16.72$, $p<0.001$; see Fig. 2e). There was also a main effect of treatment with the FGF2 animals exhibiting more USVs than the VEH animals ($F(1,40)=5.24$, $p=0.027$).

By the last day of tickling, the interaction was not significant ($F(1,39)=0.04$, $p=0.86$; see Fig. 2f). There was a main effect of phenotype on the production of 50 kHz USVs, with bHR animals exhibiting more USVs than the bLR animals ($F(1,39)=30.42$, $p<0.001$). There was also a main effect of treatment, with FGF2 animals exhibiting more USVs than vehicle-treated animals ($F(1,39)=6.59$, $p=0.014$). Overall, these results suggest that following several days of simulated social interaction, early-life FGF2 treatment improved the positive affect of both bHRs and bLRs to a similar degree.

Upon further analysis of call subtypes, we found only a little evidence that different high frequency (“50 kHz”) call subtypes were indicative of different affective responses within our paradigm. During habituation, all three subtypes of calls (40kHz, flat 50kHz and frequency-modulated (“FM”) kHz) exhibited similar effects of phenotype, treatment, and phenotype by treatment interaction, with bHR’s generally producing more calls, especially when treated with FGF2 ($p<0.057$ for all relationships, Fig. S5). During tickling, “40 kHz” and “Flat 50 kHz” calls exhibited robust effects of phenotype (bHR>bLR, $F(1,40)=8.780$, $p=0.005$; $F(1,40)=36.778$, $p<0.001$, respectively), whereas “FM 50 kHz” exhibited a main effect of treatment (FGF2>VEH, $F(1,40)=15.220$, $p<0.001$, Fig. S6). This suggests that

FGF2 may modulate the social or anticipatory aspect of tickling due to its effects on the FM USVs more so than genotype.

2.3 Early-life FGF2 treatment modulated opioid gene expression in affective circuitry

There were significant effects on the mu opioid receptor in many of the brain regions that we examined. There were four brain regions (the ACC, NAcc, BNST, and dmThal) that showed a similar pattern of expression, with the effect of FGF2 treatment dependent on the phenotype of the animal (*treatment*group*: ACC: $F(1,22)=5.40$, $p<0.05$; NAcc: $F(1,21)=17.5$, $p<0.001$; BNST: $F(1,22)=5.86$, $p<0.05$; dmThal: $F(1,21)=16.89$, $p<0.001$; see Fig. 3b). In each of these brain regions, the bLRs treated with vehicle had lower levels of mu opioid receptor expression than bHRs, and these low levels were alleviated by early-life FGF2 treatment. In all four brain regions bLR-VEH differed significantly from bHR-VEH (ACC: $p<0.05$; NAcc: $p<0.001$, BNST: $p<0.05$, dmThal: $p<0.005$), and in three of the regions bLR-VEH differed significantly from bLR-FGF2 (NAcc: $p<0.001$; BNST: $p<0.001$, dmThal: $p<0.001$). There was a significant main effect of treatment (NAcc: $F(1,21)=18.8$, $p<0.001$; BNST: $F(1,22)=18.58$, $p<0.001$; dmThal: $F(1,21)=7.33$, $p<0.05$; *no effect*: ACC: $F(1,22)=0.87$, $p=0.36$). The overall main effects of group in these four regions did not reach significance (ACC: $F(1,22)=3.12$, $p=0.09$; NAcc: $F(1,21)=3.19$, $p=0.09$; BNST: $F(1,22)=3.68$, $p=0.07$; dmThal: $F(1,21)=1.32$, $p=0.26$). Two of the other brain regions showed different trends. In the PAG, the interaction was not significant ($F(1,23)=0.03$, $p=0.87$) and neither was the main effect of treatment ($F(1,23)=0.11$, $p=0.74$). There was, however, a significant main effect of group ($F(1,23)=4.35$, $p<0.05$), with bHR animals exhibiting more mu opioid receptor expression than bLR animals. In the POA, the interaction was not significant and neither were the main effects of treatment or group in the POA. Example autoradiographs illustrating the pattern of mu receptor expression in each region can be found in the Online Resource 1: Suppl. Methods and Results.

There was only a main effect of group for the other opioid receptors, and only within a few of the brain regions. For the kappa receptor, there was no significant interaction (NAcc: $F(1,22)=1.16$, $p=0.29$; BNST: $F(1,24)=0.94$, $p=0.34$), and no significant effect of treatment (NAcc: $F(1,22)=0.61$, $p=0.44$; BNST: $F(1,24)=0.08$, $p=0.77$), as well as a lack of significant effects in any of the other brain regions (ACC, BNST, dmThal, POA, PAG). There was, however, a significant main effect of group for the kappa receptor in the NAcc and BNST, with bLRs exhibiting greater levels than bHRs (NAcc: $F(1,22)=8.90$, $p<0.05$; BNST: $F(1,24)=5.45$, $p<0.05$; see Fig. 3c). For the delta receptor, there was no significant interaction ($F(1,23)=1.73$, $p=0.20$), and no significant effect of treatment ($F(1,23)=1.16$, $p=0.29$) in the PAG, as well as a lack of significant effects in any of the other brain regions (ACC, NAcc, BNST, dmThal, POA). There was, however, a significant main effect of group for the delta receptor in the PAG, with bLRs exhibiting lower levels than bHRs ($F(1,23)=4.8$, $p<0.05$; see Fig. 3d). Example autoradiographs illustrating the pattern of kappa and delta receptor expression in each region can be seen in the Online Resource 1: Suppl. Methods and Results.

When surveying the transcripts for the opioid ligands, the effects of treatment depended on the phenotype of the animal for prodynorphin (*treatment*group*: ACC: $F(1,24)=9.65$,

$p < 0.005$; NAcc: $F(1,24)=15.24$, $p < 0.001$; BNST: $F(1,24)=14.90$, $p < 0.001$) in such a manner that early-life FGF2 decreased prodynorphin expression in bHRs and increased prodynorphin expression in bLRs (see Fig. 4b). Therefore, in many regions bHR-VEH was different from bLR-VEH (ACC: $p < 0.005$; NAcc: $p < 0.005$; BNST: $p < 0.001$), bHR-VEH was different from bHR-FGF2 (BNST: $p < 0.05$), and bLR-VEH was different from bLR-FGF2 (NAcc: $p < 0.05$). In some of the brain regions, this was accompanied by a main effect of group (ACC: $F(1,24)=6.55$, $p < 0.05$; BNST: $F(1,24)=6.42$, $p < 0.05$; *no effect*: NAcc: $F(1,22)=2.32$, $p=0.14$), but none of these regions showed an overall main effect of treatment (ACC: $F(1,24)=0.03$, $p=0.86$; NAcc: $F(1,22)=0.59$, $p=0.45$; BNST: $F(1,24)=0.003$, $p=0.96$). There were no significant effects in any of the other brain regions analyzed (dmThal, POA and PAG). Interestingly, there was a main effect of group for proenkephalin in the NAcc ($F(1,27)=6.78$, $p < 0.05$), with bLRs exhibiting more expression than bHRs (Fig. 4c), but no other significant effects. Example autoradiographs illustrating the pattern of prodynorphin and proenkephalin expression in each brain region can be found in the Online Resource 1: Suppl. Methods and Results.

3. Discussion

In three separate independent studies we documented differences in affective behavior and circuitry in selectively-bred animals, as well as their reduction following early-life treatment with FGF2. We used brief maternal separation as a way to elicit negative affect, and tickling as a way to elicit positive affect in young animals that differ in emotionality. Overall, we found that bHRs already exhibited more positive affect and somewhat less negative affect than bLRs early in development. Early-life treatment with FGF2 dampened the negative effects of maternal separation and allowed the more vulnerable bLRs to experience the initial rewarding effects of tickling. Using adult tissue, we also found that bLRs exhibited differences in the endogenous opioid system compared to bHRs. We observed alterations in opioid gene expression in many affective brain regions, especially the mu receptor. Here, we found decreased mu receptor expression in bLRs compared to bHRs across several regions, and FGF2 treatment alleviated the low levels in bLRs. FGF2 also increased the expression of the transcript for one of the opioid ligands, prodynorphin, in bLRs. Transcripts for other opioid receptors and ligands showed basal differences between bHRs and bLRs but no effect of FGF2. Taken together, these results suggest that FGF2 dampens negative emotional responses and enhances positive emotional responses in vulnerable animals. FGF2 also alters the endogenous opioid system in a manner that is likely to alter affect. Further studies should assess the effects of FGF2 on the opioid system at earlier timepoints to characterize the developmental emergence of these differences and relevance to affective behavior.

By measuring USVs, we found that differences in affect emerged as early as the post-natal and juvenile periods in our selectively-bred HR/LR model. Perhaps most importantly, early-life treatment with FGF2 was able to alter the behavior of the bLR animals. Early-life FGF2 treatment decreased the USV distress response to maternal separation in bLRs in a manner similar to what was previously observed in outbred rats (Litvin et al. 2016). Juvenile expression of positive affect similarly differed. bHRs exhibited more 50 kHz USVs than bLRs during habituation to a novel environment, and FGF2 generally increased 50 kHz USV production, suggesting an increase in positive affect during habituation. This is important

because habituation results are the closest measurement of a basal state when examining USVs, given that some manipulation is typically necessary to elicit USVs. On the first day of tickling, bHRs exhibited more 50 kHz USVs than bLRs. In contrast, FGF2 increased the number of 50 kHz USVs only in bLRs. This suggests that FGF2 can increase positive affect in the initial response to tickling, a naturally rewarding stimuli, in animals that exhibit less positive affect. On the last day of tickling, FGF2 increased 50 kHz USVs overall without a selective effect on bLRs. bHRs still had more USVs than bLRs. Thus, early-life FGF2 may improve affect, especially in the bLRs that had high negative affect and low positive affect. From a translational standpoint, this is important given that positive affective states can protect against depression and anxiety (Fredrickson et al. 2003; Lyubomirsky et al. 2005).

Using adult tissue, we found that opioid gene expression was modulated by FGF2 in a manner consistent with decreasing negative affect or distress within brain regions that have also been previously associated with the production of emotional USVs (Akil et al. 1984; Herman and Panksepp 1978; Panksepp et al. 1978). The mu opioid receptor is important for the reduction of separation distress vocalizations (Carden et al. 1991; Moles et al. 2004) and has been generally associated with positive affect (Garcia-Horsman et al. 2008; Kelley et al. 2002; Zubieta et al. 2003). Across multiple brain regions, mu receptors were decreased in bLRs compared to bHRs, and FGF2 increased mu receptor expression in bLRs. These results are particularly interesting in the light of data implicating the mu receptor as a target for tianeptine, an atypical antidepressant which has similar efficacy to the selective serotonin reuptake inhibitors fluoxetine and sertraline (Gassaway et al. 2014).

In contrast, delta and kappa opioid receptor expression differed with bLR/bHR phenotype but were unaffected by FGF2 treatment. bLRs had more kappa expression than bHRs. This pattern aligns with the known effects of kappa receptor activation increasing anxiety-like behavior (Knoll et al. 2007; Marin et al. 2003), separation distress (Carden et al. 1991) and negative affect (Carr and Bak 1988; Garcia-Horsman et al. 2008; Kelley et al. 2002; Warnick et al. 2005). bLRs had less delta receptor expression than bHRs. This mirrors previous studies showing that activation of delta opioid receptors using pharmacological agonists reduces separation distress calls (Carden et al. 1991). Delta receptors are also among the most consistent targets for novel antidepressant drugs (Lutz and Kieffer 2013; Torregrossa et al. 2005; Torregrossa et al. 2004; Torregrossa et al. 2006; Zhang et al. 2006).

One of the primary transcripts for the endogenous opioid ligands, prodynorphin, also showed differences in expression related to phenotype in some regions. Here, bLR-VEH animals had less gene expression than bHR-VEH animals in a manner that replicated what had been previously observed in the nucleus accumbens in outbred HR and LR rats (Lucas et al., 1998). FGF2 acted as an equalizer between bHRs and bLRs. In this regard, FGF2 decreased prodynorphin expression in bHRs and increased prodynorphin expression in bLRs. Prodynorphin is post-translationally processed into opioid ligands that are either kappa or mu/delta preferring (Mansour et al. 1995), and further work is needed to ascertain the final products within these affective brain regions in bHRs versus bLRs. However, basal differences in prodynorphin expression and its differential regulation by FGF2 further support a role for the opioid system in regulating affect.

Taken together, these results indicate that opioid gene expression is different in animals that differ in emotionality, and hence may play a role in affect regulation. Similarly, early-life administration of FGF2 produces effects in the opioid system that are measurable in adulthood, analogous to the effects of early-life FGF2 administration on adult anxiety (Litvin et al. 2016; Prater et al. 2017; Turner et al. 2011). Since opioid signaling in these regions has been linked to the production of USVs in the early postnatal and juvenile periods, these results beg the question as to whether the effects of early-life FGF2 administration on the opioid system emerge early in life and mediate the effects that we observed on negative and positive USV responses. This question could be addressed in future studies using measurements in younger animals following FGF2 treatment, preferably while directly modulating opioid function.

The discovery of basal differences in opioid gene expression in bLR and bHR rats may also be of interest because the LR/HR model is commonly used to research individual differences in susceptibility to drug addiction in addition to individual differences in anxiety- and depression-like behavior (Flagel et al., 2010; Piazza et al., 1989). Previous studies using outbred rats with high and low responses to novelty have indicated that HR rats are more sensitive to the psychomotor effects of morphine (Deroche et al., 1993). Since morphine preferentially binds to the mu receptor, our results demonstrating elevated expression of mu receptor expression across multiple affective brain regions, including the nucleus accumbens, may provide insight into differences in drug sensitivity in the HR/LR model.

An interesting question raised by our study is whether the differences in affective behavior and circuitry that we observed emerged directly from bHR/bLR genotype or due to indirect influences on maternal care (Francis et al., 1999). It is possible that maternal behavior plays a role; however, in previous cross-fostering studies maternal phenotype had only a modest influence on anxiety-like behavior in bLR rats (Stead et al., 2006), most likely due to the overwhelming effect of multiple generations of selective breeding. Likewise, although it is possible that early-life FGF2 administration may be altering the manner with which the pups solicit and receive care from the dams, previous studies have indicated that maternal behavior in our model is relatively robust to behavior differences in the pups (Clinton et al. 2010).

There are limitations in our work that could be addressed by future studies. The generalizability of the results are limited to males, as we did not collect data from females. This is an oversight on our part, as females exhibit a higher incidence of depression and anxiety disorders than males. Furthermore, our studies directly followed-up on our previous work on FGF2-administration in outbred and selectively-bred rats (Chaudhury et al. 2014; Litvin et al. 2016; Turner et al. 2011), and therefore used the same dosage as the previous studies, but we recognize the utility of measuring a more detailed dose-response curve. Finally, we did not examine specific pharmacological mechanisms in this paper. This would be a fascinating area for future research, as the mu receptor is known to interact indirectly with FGF receptors (Di Liberto et al. 2014). The mu receptor can transactivate FGF receptors by PKC-mediated MMP activation. The MMP activation results in cleavage of FGF2 (coupled to heparin sulfate) thereby increasing FGF2 levels and receptor activation. Although this is an indirect transactivation, we have observed increased FGF2 expression in

bHR animals, the same animals that show increased mu receptor basally (Eren-Kocak et al. 2011; Perez et al. 2009).

In conclusion, affective valence is fundamentally different in bLRs and bHRs, and this can be seen very early in development: bHRs exhibit somewhat less negative affect and significantly more positive affect than bLRs. However, early-life FGF2 can decrease negative affect and increase positive affect in the vulnerable bLRs. Moreover, both phenotype and treatment was associated with alterations in mu opioid receptor gene expression in multiple brain regions responsible for the production of USVs and affect regulation. This increase in mu receptor expression is likely to assuage separation distress and enhance playful affiliative behavior (Machin AJ 2011; Panksepp et al. 1980), although future studies still need to ascertain whether the effects of early-life FGF2 on the opioid system manifest during early development. Likewise, although the exact mechanism by which neonatal FGF2 treatment is modifying opioid system expression in these regions is unknown, the literature suggests that pharmacological agents working on mu opioid receptors may activate the same intracellular signaling pathways that are downstream of FGF receptors (Persson et al. 2003a; Persson et al. 2003b), suggesting that these molecular pathways may converge. Future work should more directly assess the interplay between FGF2 and opioid function. Finally, since mu receptor activation has also been implicated in the action of the antidepressant tianeptine (Gassaway et al. 2014), our results demonstrating large differences in opioid gene expression between our selectively bred lines suggest that it may be fruitful to explore the relationship between antidepressant effectiveness and basal variation in the FGF and opioid systems.

4. Experimental Procedure

4.1 General Methods

4.1.1 Animals.—We used selectively-bred bHR and bLR male rats from generations F30-F37. Similar to previous studies, naïve bHR and bLR rats were bred, weaned, and tested for their locomotor response to a novel environment between postnatal days 50–60, and housed in pairs in-house, as previously described (Stead et al. 2006). Depending on the generation, bHRs rats had a high response to a novel environment (1682–2094 average beam breaks), whereas bLR rats had a low response to a novel environment (50–89 average beam breaks). All animals were maintained on a 12:12 light:dark schedule, with access to food and water *ad libitum*. All animals were treated in accordance with the National Institutes of Health *Guidelines on Laboratory Animal Use and Care* and in accordance with the guidelines set by the University Committee on Use and Care of Animals at the University of Michigan.

4.1.2 Drug.—To test the effects of early-life FGF2 administration, animals were injected with either FGF2 (20 ng/g in 50 µl of 0.1% BSA, s.c; Sigma-Aldrich) or vehicle (VEH; 0.1M PBS with 0.1% BSA, s.c.) on the day after birth (PND1) with each group consisting of animals from multiple litters (Turner et al. 2011). Rats were then housed until either behavioral testing or brain collection without further intervention or drug administration. Rats were not selectively bred after drug administration.

4.2 Experiment 1: USV Response to Maternal Separation in Early-life

4.2.1 Animals: We examined the effect of early-life FGF2 treatment on negative affect on PND11 in bHR and bLR rats by measuring USVs in response to separation from the dam. The animals were from generation F37. The number of animals per group was as follows: bHR-VEH (n=15), bLR-VEH (n=14), bHR-FGF2 (n=11), bLR-FGF2 (n=14).

4.2.2 Testing Procedure: All pups within a litter were removed from the dam and transferred to a dimly-lit separate testing room at least 30 minutes prior to testing and acclimated to a 32° C digital moist heating pad (ReliaMed). Each pup was tested for USVs individually in a novel chamber (clean housing cage lined with digital moist heating pad at 32°C with a layer of corncob bedding) for five minutes. During this time, 40 kHz USVs were detected using the UltraSoundGate condenser microphone NCMXHD (Avisoft, Bioacoustics, Germany). The microphone was routed through an Ultrasoundgate amplifier (416H, gain set at 5.5 notches) connected by a USB Audio Device to a computer that contained Avisoft-Recorder USGH Software (Avisoft Bioacoustics). USVs were recorded within a range of 20–120 kHz, with a sample rate of 250 kHz, and the total number of 40 kHz USVs were counted manually by an observer blind to the condition of the animal.

4.2.3 Statistics: All data were analyzed in SPSSv.24 (IBM, Armonk, NY) by a two-way ANOVA (phenotype × treatment). If the interaction was significant, a One-way ANOVA followed by Tukey honest significant difference comparisons was performed. Data are presented as means and S.E.M.

4.3 Experiment 2: USV Response to Heterospecific Hand Play and Exploration in Juveniles

4.3.1 Animals and Testing Procedure: We examined the effects of early-life FGF2 treatment on positive affect in bHR and bLR rats in response to heterospecific hand-play or “tickling” during the juvenile period. Animals were from generation F33. The animals were weaned on postnatal day 21 and singly-housed for the remainder of the experiment. Under dim light (<40 lux) on PND22, bHR-VEH (n=10), bHR-FGF2 (n=10), bLR-VEH (n=11) and bLR-FGF2 (n=13) animals were habituated to one minute of handling and two minutes in the testing arena, which consisted of a clean housing cage without bedding, lined with a fresh piece of thin carpet to reduce sound reverberation. On PND23–26, animals were tickled once per day over a period of two minutes in alternating 15-second blocks (“OFF” for 15 seconds and “ON” for 15 seconds, repeated four times) by an experimenter that was blind to the experimental condition of the animal. Tickling consisted of dorsal contacts and gentle ventral rubbing with the tips of the fingers taking care not to injure the animal (for additional methodological detail, see (Burgdorf and Panksepp 2001)). Ultrasonic vocalizations were recorded as described above. In this experiment, ultrasonic vocalizations were recorded within a range of 0–250 kHz, with a sample rate of 250 kHz, but only vocalizations within a range of 15–120 kHz were considered in later analyses (described below).

4.3.2 Quantification of USVs: The ultrasonic vocalizations from the habituation and tickling sessions were characterized using a set of automated analyses within Avisoft

SASlab Pro software (Avisoft Bioacoustics). In order to best detect quick frequency modulations, audio files (.wav) were loaded with a bandwidth of 3672 Hz and a resolution of 976 Hz. These files were then converted to a standard spectrogram, with time on the x-axis (in 50 msec increments), frequency on the y-axis (“pitch”: 15–120 kHz), and the intensity of the display indicative of amplitude (“loudness”: range: –77 to –40 dB). Low frequency (“22 kHz”) distress calls were counted by hand, and were characterized by low frequencies (<30 kHz), long duration (often >200 msec), and almost complete lack of frequency modulation (“flat”). The high pass filter for the spectrogram was then reset to 30 kHz to eliminate low frequency noise before performing an automated analysis of higher frequency (“50 kHz”) calls. We detected high frequency calls using the “automatic whistle tracking” setting, which uses an algorithm that searches for steady signals that have a relatively stable (peak) frequency course without extremely rapid/dramatic frequency modulations (<19.06 kHz/msec). This setting assumes that background noise will have a broadband structure (i.e., occupy many different frequencies simultaneously). These call elements were then further separated by defining five msec as the minimum call-element duration, and specifying that elements occurring within 30 msec of each other would be defined as part of a single multi-component call (“hold time”). These calls were also post-filtered to reject calls of <8 msec total duration and >0.5 maximum entropy. Using these automated analysis settings; we found that 640 out of a sample of 661 hand-counted calls were accurately detected, with 0 cases of misidentified noise, 21 missed calls, and 3 falsely-divided multi-component calls (i.e., an overall accuracy of 97%). We performed further analyses based on call-type (e.g., flat vs. frequency-modulated), but found that the results were relatively consistent across all high frequency call types (Online Resource 1: Suppl. Methods and Results). All high frequency call subtypes also correlated similarly with anticipatory behaviors recorded between tickling sessions (Online Resource 1: Suppl. Methods and Results).

4.3.3 Statistics: Two-way ANOVAs were used to examine the effect of phenotype, treatment and phenotype $\times$ treatment on USV production using IBM SPSS Statistics (Armonk, NY), applying Tukey HSD (honestly significant difference) multiple comparisons when appropriate. Call characterization and filtering were performed in R using available base packages.

4.4 Experiment 3: Opioid System Gene Expression in Affective Circuitry

4.4.1 Animals: Early-life FGF2 was administered to bHR and bLR rats from generation F30 on PND1 (n=8 per group: bHR-VEH, bHR-FGF2, bLR-VEH, bLR-FGF2) similar to other experiments. Animals were weaned at PND21, pair-housed until PND56, and then sacrificed by rapid decapitation in the morning. Brains were removed, snap-frozen in 2-methylbutane, and stored at –80C until processing.

4.4.2 mRNA in situ hybridization: Coronal sections (10 μ m) were taken every 100–200 μ m (depending on the region) and mounted onto Superfrost Plus slides at –20°C (Fisher, Waltham, MA). Slide-mounted tissue was fixed in 4% paraformaldehyde solution for 60 minutes, washed three times with 2X SSC (1X SSC is 150 mM sodium chloride and 15 mM sodium citrate), and treated with 0.1M triethanolamine with 0.25% acetic anhydride. Slides were rinsed and dehydrated in graded ethanols before air-drying. All *in situ* probes were

synthesized in-house. All cDNA segments were extracted (Qiaquick Gel Extraction kit, Qiagen, Valencia, CA), subcloned in Bluescript SK (Stratagene, La Jolla, CA) and confirmed by nucleotide sequencing. The sequences for generating the probes are as follows: mu receptor (L22455; pos. 663–1034), delta receptor (U00475; pos. 247–1230), kappa receptor (U00442; pos. 1350–2130), prodynorphin (M32784; pos. 841–1574), proenkephalin (K02807; pos. 201–890). The probes were labeled in a reaction mixture of 0.5–1 µg of linearized plasmid specific to the probe of interest, 1X transcription buffer (Epicentre Technologies, Madison, WI), 125 µCi of 35S-labeled UTP, 125 µCi of 35-S labeled CTP, 150 µM ATP and GTP, 12.5 mM dithiothreitol, 1 µl of RNase inhibitor, and 1.5 µl of T7 or T3 RNA polymerase. Labeled probes were purified on Micro Bio-Spin Chromatography Columns (BioRad, Berkeley, CA) according to the manufacturer's instructions. After air-drying, slides were treated with hybridization buffer containing the labeled probe ($1-2 \times 10^6$ counts/75 µL buffer) 50% formamide, 10% dextran sulfate, 3X SSC, 50 mM sodium phosphate buffer (pH = 7.4), 1X Denhardt's solution, 0.1 mg/ml yeast tRNA, and 10 mM dithiothreitol. All slides were cover-slipped and stored in humidified chambers at 55°C during the 12–16 hour hybridization period. After hybridization, sections were washed three times in 2X SSC and incubated in an RNase solution (100 µg/mL RNase in Tris buffer with 0.5M NaCl, pH=8) at 37°C. Sections were then sequentially washed in 2X, 1X, and 0.5X SSC before being incubated in 0.1X SSC at 65°C for 1 hour. Sections were rinsed in distilled water and dehydrated through graded ethanols. Slides were exposed to Kodak BioMax MR Scientific Imaging Film (Sigma Aldrich), and exposure times were experimentally determined for optimal signal, as follows: proenkephalin (1 day), prodynorphin (7 days), mu receptor (7 days), kappa receptor (21 days) and delta receptor (14 days).

4.4.3 mRNA in situ hybridization quantification: mRNA expression signals from autoradiographic films were quantified using the computer-assisted optical densitometry software ImageJ (National Institutes of Health, Bethesda, MDD). We defined each region using custom-made templates that can be viewed in the Online Resource 1: Suppl. Methods and Results: the anterior cingulate cortex (ACC), nucleus accumbens (NAcc), preoptic area (POA), bed nucleus of the stria terminalis (BNST), mediodorsal thalamus (dmThal), or periaqueductal grey (PAG). These templates were created by tracing the morphology of a region as defined by probes that were expressed in that region (NAcc: D1 dopamine receptor; POA: Galanin; rostral BNST: Enkephalin; caudal BNST: cocaine and amphetamine regulated transcript; PAG: Mu opioid receptor) or by probes that were not expressed in the region (dmThal: 5HT-7 serotonin receptor). We defined coordinates from Bregma for each brain region coordinates for quantification as follows: ACC: +1.2 to +0.2; NAcc: +1.2 to +0.2; POA: -0.27 to -0.4; BNST: +0.2 to -0.26 and -0.3 to -0.4; dmThal: -1.8 to -1.88 and -2.12 to -3.3; PAG: -5.2 to -6.04 and -6.3 to -8.0 (Paxinos and Watson 1998). Measurements were collected for each hemisphere, correcting for background plus 3.5 times its standard deviation. Data from >6 sections were averaged to create a mean signal per animal and group averages and standard error of the mean were calculated.

4.4.4 Statistics: All data were analyzed in SPSSv.21 (IBM, Armonk, NY) by a two-way ANOVA (phenotype $\times$ group), applying Tukey HSD (honestly significant difference) multiple comparisons when appropriate. Data are presented as means and S.E.M.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by National Institute of Mental Health (NIMH) Conte Center Grant P50 MH60398, National Institute on Drug Abuse (NIDA) P01 DA021633, The Office of Naval Research (ONR) Grants N00014-09-1-0598 and N00014-12-1-0366, the Pritzker Neuropsychiatric Disorders Research Consortium Fund LLC (<http://www.pritzkerneuropsych.org>), the Hope for Depression Research Foundation, National Institutes of Health (NIH) NCRR Grant UL1RR024986, as well as a Rachel Upjohn Clinical Scholars Award (CT), National Institutes of Health (NIH) Training Grant T32-DK071212 (MHH), National Science Foundation Graduate Research Fellowship Program (ELA), and Undergraduate Research Opportunities Program of the University of Michigan (CF, RC). The authors would like to further thank Angela Koelsch, Jim Stewart, Hui Li, Jennifer Fitzpatrick, and Fei Li for their careful animal husbandry and expert assistance in laboratory techniques, and previous reviewers for their expert feedback on our manuscript.

Declarations of Interest

The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, the University of California at Irvine, and the HudsonAlpha Institute for Biotechnology to encourage the development of appropriate findings for research and clinical applications.

Abbreviations

bHR	A rat that is selectively bred to be highly exploratory in a novel environment. Prone to impulsive, aggressive, and reward-seeking behavior.
bLR	A rat that is selectively bred to be highly inhibited in a novel environment. Extremely anxious and prone to developing depressive-like behavior.
FGF2	Fibroblast growth factor-2. A growth factor that has been previously shown to have antidepressant and anxiolytic effects in animals when administered either in early life or adulthood.
PND	Postnatal day, number of days since birth.
USVs	Ultrasonic vocalizations, one of the clearest measures of positive and negative affect in rodents.
FM	Frequency-modulated ultrasonic vocalizations
POA	Preoptic area
BNST	Bed nucleus of the stria terminalis
dmThal	Dorsomedial thalamus
NAcc	Nucleus accumbens
PAG	Periaqueductal gray

ACC Cingulate cortex

References

- Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM (1984) Endogenous opioids: biology and function. *Annu Rev Neurosci* 7: 223–55. [PubMed: 6324644]
- Berrocchio E, Mico JA (2009) Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. *Int J Neuropsychopharmacol* 12: 1033–44. [PubMed: 19341511]
- Burgdorf J, Colechio EM, Ghoreishi-Haack N, Gross AL, Rex CS, Zhang XL, Stanton PK, Kroes RA, Moskal JR (2017) IGFBP2 Produces Rapid-Acting and Long-Lasting Effects in Rat Models of Posttraumatic Stress Disorder via a Novel Mechanism Associated with Structural Plasticity. *Int J Neuropsychopharmacol* 20: 476–484. [PubMed: 28158790]
- Burgdorf J, Knutson B, Panksepp J, Ikemoto S (2001a) Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behav Neurosci* 115: 940–4. [PubMed: 11508733]
- Burgdorf J, Knutson B, Panksepp J, Shippenberg TS (2001b) Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs. *Psychopharmacology (Berl)* 155: 35–42. [PubMed: 11374334]
- Burgdorf J, Kroes RA, Beinfeld MC, Panksepp J, Moskal JR (2010) Uncovering the molecular basis of positive affect using rough-and-tumble play in rats: a role for insulin-like growth factor I. *Neuroscience* 168: 769–77. [PubMed: 20350589]
- Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, Brudzynski SM, Panksepp J (2008) Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. *J Comp Psychol* 122: 357–67. [PubMed: 19014259]
- Burgdorf J, Panksepp J (2001) Tickling induces reward in adolescent rats. *Physiol Behav* 72: 167–73. [PubMed: 11239994]
- Burgdorf J, Panksepp J, Moskal JR (2011) Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci Biobehav Rev* 35: 1831–6. [PubMed: 21144859]
- Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J (2007) Neurobiology of 50-kHz ultrasonic vocalizations in rats: electrode mapping, lesion, and pharmacology studies. *Behav Brain Res* 182: 274–83. [PubMed: 17449117]
- Carden SE, Barr GA, Hofer MA (1991) Differential effects of specific opioid receptor agonists on rat pup isolation calls. *Brain Res Dev Brain Res* 62: 17–22. [PubMed: 1662121]
- Carr KD, Bak TH (1988) Medial thalamic injection of opioid agonists: mu-agonist increases while kappa-agonist decreases stimulus thresholds for pain and reward. *Brain Res* 441: 173–84. [PubMed: 2833999]
- Chaudhury S, Aurbach EL, Sharma V, Blandino P Jr., Turner CA, Watson SJ, Akil H (2014) FGF2 is a target and a trigger of epigenetic mechanisms associated with differences in emotionality: partnership with H3K9me3. *Proc Natl Acad Sci U S A* 111: 11834–9. [PubMed: 25071177]
- Clinton SM, Bedrosian TA, Abraham AD, Watson SJ, Akil H (2010) Neural and environmental factors impacting maternal behavior differences in high- versus low-novelty-seeking rats. *Horm Behav* 57: 463–73. [PubMed: 20156440]
- Clinton SM, Vazquez DM, Kabbaj M, Kabbaj MH, Watson SJ, Akil H (2007) Individual differences in novelty-seeking and emotional reactivity correlate with variation in maternal behavior. *Horm Behav* 51: 655–64. [PubMed: 17462647]
- Di Liberto V, Mudo G, Fuxe K, Belluardo N (2014) Interactions between cholinergic and fibroblast growth factor receptors in brain trophism and plasticity. *Curr Protein Pept Sci* 15: 691–702. [PubMed: 25175454]
- Elsayed M, Banasr M, Duric V, Fournier NM, Licznarski P, Duman RS (2012) Antidepressant Effects of Fibroblast Growth Factor-2 in Behavioral and Cellular Models of Depression. *Biol Psychiatry*.

- Eren-Kocak E, Turner CA, Watson SJ, Akil H (2011) Short-hairpin RNA silencing of endogenous fibroblast growth factor 2 in rat hippocampus increases anxiety behavior. *Biol Psychiatry* 69: 534–40. [PubMed: 21215386]
- Fredrickson BL, Tugade MM, Waugh CE, Larkin GR (2003) What good are positive emotions in crises? A prospective study of resilience and emotions following the terrorist attacks on the United States on September 11th, 2001. *J Pers Soc Psychol* 84: 365–76. [PubMed: 12585810]
- Garcia-Horsman SP, Agmo A, Paredes RG (2008) Infusions of naloxone into the medial preoptic area, ventromedial nucleus of the hypothalamus, and amygdala block conditioned place preference induced by paced mating behavior. *Horm Behav* 54: 709–16. [PubMed: 18721808]
- Gassaway MM, Rives ML, Kruegel AC, Javitch JA, Sames D (2014) The atypical antidepressant and neurorestorative agent tianeptine is a mu-opioid receptor agonist. *Transl Psychiatry* 4: e411. [PubMed: 25026323]
- Herman BH, Panksepp J (1978) Effects of morphine and naloxone on separation distress and approach attachment: evidence for opiate mediation of social affect. *Pharmacol Biochem Behav* 9: 213–20. [PubMed: 568801]
- Hoshaw BA, Malberg JE, Lucki I (2005) Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Res* 1037: 204–8. [PubMed: 15777771]
- Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M (2002) Opioid modulation of taste hedonics within the ventral striatum. *Physiol Behav* 76: 365–77. [PubMed: 12117573]
- Knoll AT, Meloni EG, Thomas JB, Carroll FI, Carlezon WA Jr., (2007) Anxiolytic-like effects of kappa-opioid receptor antagonists in models of unlearned and learned fear in rats. *J Pharmacol Exp Ther* 323: 838–45. [PubMed: 17823306]
- Knutson B, Burgdorf J, Panksepp J (1999) High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats. *Physiol Behav* 66: 639–43. [PubMed: 10386908]
- Knutson B, Burgdorf J, Panksepp J (2002) Ultrasonic vocalizations as indices of affective states in rats. *Psychol Bull* 128: 961–77. [PubMed: 12405139]
- Litvin Y, Turner CA, Rios MB, Maras PM, Chaudhury S, Baker MR, Blandino P Jr., Watson SJ Jr., Akil H, McEwen B (2016) Fibroblast growth factor 2 alters the oxytocin receptor in a developmental model of anxiety-like behavior in male rat pups. *Horm Behav* 86: 64–70. [PubMed: 27693608]
- Lutz PE, Kieffer BL (2013) Opioid receptors: distinct roles in mood disorders. *Trends Neurosci* 36: 195–206. [PubMed: 23219016]
- Lyubomirsky S, King L, Diener E (2005) The benefits of frequent positive affect: does happiness lead to success? *Psychol Bull* 131: 803–55. [PubMed: 16351326]
- Machin AJ DR (2011) The brain opioid theory of social attachment: a review of the evidence. *Behaviour* 148: 985–1025.
- Mansour A, Hoversten MT, Taylor LP, Watson SJ, Akil H (1995) The cloned mu, delta and kappa receptors and their endogenous ligands: evidence for two opioid peptide recognition cores. *Brain Res* 700: 89–98. [PubMed: 8624732]
- Marin S, Marco E, Biscaia M, Fernandez B, Rubio M, Guaza C, Schmidhammer H, Viveros MP (2003) Involvement of the kappa-opioid receptor in the anxiogenic-like effect of CP 55,940 in male rats. *Pharmacol Biochem Behav* 74: 649–56. [PubMed: 12543231]
- Moles A, Kieffer BL, D'Amato FR (2004) Deficit in attachment behavior in mice lacking the mu-opioid receptor gene. *Science* 304: 1983–6. [PubMed: 15218152]
- Panksepp J (2003) Neuroscience. Feeling the pain of social loss. *Science* 302: 237–9. [PubMed: 14551424]
- Panksepp J (2005) Why does separation distress hurt? Comment on MacDonald and Leary (2005). *Psychol Bull* 131: 224–30; author reply 237–40. [PubMed: 15740418]
- Panksepp J, Herman BH, Vilberg T, Bishop P, DeEsquinazi FG (1980) Endogenous opioids and social behavior. *Neurosci Biobehav Rev* 4: 473–87. [PubMed: 6258111]
- Panksepp J, Nelson E, Bekkedal M (1997) Brain systems for the mediation of social separation-distress and social-reward. Evolutionary antecedents and neuropeptide intermediaries. *Ann N Y Acad Sci* 807: 78–100. [PubMed: 9071345]

- Panksepp J, Normansell L, Herman B, et al. (1988) Neural and Neurochemical Control of the Separation Distress Call The Physiological Control of Mammalian Vocalization. Springer, Boston, MA, pp 263–299
- Panksepp J, Vilberg T, Bean NJ, Coy DH, Kastin AJ (1978) Reduction of distress vocalization in chicks by opiate-like peptides. *Brain Res Bull* 3: 663–7. [PubMed: 318190]
- Paxinos G, Watson C (1998) *Stereotaxic Brain Atlas*. Academic Press, New York
- Perez JA, Clinton SM, Turner CA, Watson SJ, Akil H (2009) A new role for FGF2 as an endogenous inhibitor of anxiety. *J Neurosci* 29: 6379–87. [PubMed: 19439615]
- Persson AI, Thorlin T, Bull C, Eriksson PS (2003a) Opioid-induced proliferation through the MAPK pathway in cultures of adult hippocampal progenitors. *Mol Cell Neurosci* 23: 360–72. [PubMed: 12837621]
- Persson AI, Thorlin T, Bull C, Zarnegar P, Ekman R, Terenius L, Eriksson PS (2003b) Mu- and delta-opioid receptor antagonists decrease proliferation and increase neurogenesis in cultures of rat adult hippocampal progenitors. *Eur J Neurosci* 17: 1159–72. [PubMed: 12670304]
- Prater KE, Aurbach EL, Larcinese HK, Smith TN, Turner CA, Blandino P Jr., Watson SJ, Maren S, Akil H (2017) Selectively Bred Rats Provide a Unique Model of Vulnerability to PTSD-Like Behavior and Respond Differentially to FGF2 Augmentation Early in Life. *Neuropsychopharmacology* 42: 1706–1714. [PubMed: 28205604]
- Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* 22: 3251–61. [PubMed: 11943826]
- Stead JD, Clinton S, Neal C, Schneider J, Jama A, Miller S, Vazquez DM, Watson SJ, Akil H (2006) Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. *Behav Genet* 36: 697–712. [PubMed: 16502134]
- Torreghrossa MM, Folk JE, Rice KC, Watson SJ, Woods JH (2005) Chronic administration of the delta opioid receptor agonist (+)BW373U86 and antidepressants on behavior in the forced swim test and BDNF mRNA expression in rats. *Psychopharmacology (Berl)* 183: 31–40. [PubMed: 16220339]
- Torreghrossa MM, Isgor C, Folk JE, Rice KC, Watson SJ, Woods JH (2004) The delta-opioid receptor agonist (+)BW373U86 regulates BDNF mRNA expression in rats. *Neuropsychopharmacology* 29: 649–59. [PubMed: 14647482]
- Torreghrossa MM, Jutkiewicz EM, Mosberg HI, Balboni G, Watson SJ, Woods JH (2006) Peptidic delta opioid receptor agonists produce antidepressant-like effects in the forced swim test and regulate BDNF mRNA expression in rats. *Brain Res* 1069: 172–81. [PubMed: 16364263]
- Turner CA, Clinton SM, Thompson RC, Watson SJ Jr., Akil H (2011) Fibroblast growth factor-2 (FGF2) augmentation early in life alters hippocampal development and rescues the anxiety phenotype in vulnerable animals. *Proc Natl Acad Sci U S A* 108: 8021–5. [PubMed: 21518861]
- Turner CA, Gula EL, Taylor LP, Watson SJ, Akil H (2008) Antidepressant-like effects of intracerebroventricular FGF2 in rats. *Brain Res* 1224: 63–8. [PubMed: 18586016]
- Vivian JA, Miczek KA (1993) Diazepam and gepirone selectively attenuate either 20–32 or 32–64 kHz ultrasonic vocalizations during aggressive encounters. *Psychopharmacology (Berl)* 112: 66–73. [PubMed: 7871011]
- Warnick JE, McCurdy CR, Sufka KJ (2005) Opioid receptor function in social attachment in young domestic fowl. *Behav Brain Res* 160: 277–85. [PubMed: 15863224]
- Zhang H, Torreghrossa MM, Jutkiewicz EM, Shi YG, Rice KC, Woods JH, Watson SJ, Ko MC (2006) Endogenous opioids upregulate brain-derived neurotrophic factor mRNA through delta- and micro-opioid receptors independent of antidepressant-like effects. *Eur J Neurosci* 23: 984–94. [PubMed: 16519663]
- Zubieta JK, Ketter TA, Bueller JA, Xu Y, Kilbourn MR, Young EA, Koeppe RA (2003) Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. *Arch Gen Psychiatry* 60: 1145–53. [PubMed: 14609890]

Highlights:

- Selectively-bred high- and low-responder rats differed in affect in early life
- High- and low-responder rats showed different opioid gene expression
- Neonatal administration of FGF2 decreased maternal separation distress calls at P11
- Neonatal FGF2 increased positive affect during exploration and play in juveniles
- Neonatal FGF2 altered expression of the mu opioid receptor and prodynorphin

Fig. 1. Early-life FGF2 decreased maternal separation-induced 40 kHz USVs in bLRs.

a) An illustration of the experimental design. **b)** Distress USVs in the bLR animals were reduced by neonatal treatment with FGF2 * $p < 0.05$.

Fig. 2. Early-life FGF2 increased positive affect (50 kHz USVs) in juvenile bHRs and bLRs during exploration and tickling.

a) An illustration of the experimental design. **b)** During habituation to the novel testing environment, bHRs exhibited more 50 kHz USVs than bLRs. FGF2 increased the number of 50 kHz USVs compared to vehicle. bHR-FGF2 animals had more USVs than bHR-VEH, bLR-VEH or bLR-FGF2 animals. *** $p < 0.001$. **c)** On Day 1 of tickling, bHRs exhibited more 50 kHz USVs than bLRs. bLR-VEH animals exhibited less 50 kHz USVs than bLR-FGF2 or bHR-VEH animals. * $p < 0.05$, ** $p < 0.005$. **d)** On Day 2 of tickling, bHRs exhibited more 50 kHz USVs than bLRs. *** $p < 0.001$. **e)** On Day 3 of tickling, bHRs exhibited more 50 kHz USVs than bLRs. *** $p < 0.001$. **f)** On Day 4 of tickling, bHRs exhibited more 50 kHz USVs than bLRs. *** $p < 0.001$.

kHz USVs than bLRs. FGF2 animals exhibited more USVs than VEH animals. * $p < 0.05$. *** $p < 0.001$. **f**) On Day 4 of tickling, bHRs exhibited more 50 kHz USVs than bLRs, and FGF2 increased the number of 50 kHz USVs compared to vehicle animals. * $p < 0.05$, *** $p < 0.001$.

Fig. 3. Effect of early-life FGF2 on opioid receptor expression in affective brain regions.

a) An illustration of the experimental design. **b)** Basal expression in the mu opioid receptor differs between bHR and bLR rats across affective brain regions, but early-life treatment with FGF2 eliminates these differences (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$; ACC: bHR-VEH vs. bLR-VEH: $p < 0.05$; NAcc: bHR-VEH vs. bLR-VEH: $p < 0.001$, bLR-FGF2 vs. bLR-VEH: $p < 0.001$; BNST: bHR-VEH vs. bLR-VEH: $p < 0.05$, bLR-FGF2 vs. bLR-VEH: $p < 0.001$; dmThal: bHR-VEH vs. bLR-VEH: $p < 0.005$, bLR-FGF2 vs. bLR-VEH: $p < 0.001$; PAG: bHRs vs. bLRs: $p < 0.05$). **c)** bLRs had more kappa receptor expression than bHRs

(* $p < 0.05$; *NAcc*: $p < 0.05$; *BNST*: $p < 0.05$). **d)** In the PAG, bHRs had more delta receptor expression than bLRs (* $p < 0.05$).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 4. Effect of early-life FGF2 on prodynorphin and proenkephalin expression in affective brain regions.

a) An illustration of the experimental design. **b)** Pro-dynorphin was basally expressed at higher levels in bHRs, and early-life treatment with FGF2 had phenotype-specific effects to eliminate these differences in NAcc (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$; ACC: bHR-VEH vs. bLR-VEH: $p < 0.005$; NAcc: bHR-VEH vs. bLR-VEH: $p < 0.005$, bLR-FGF2 vs. bLR-VEH: $p < 0.05$; BNST: bHR-VEH vs. bLR-VEH: $p < 0.001$; bHR-FGF2 vs. bHR-VEH: $p < 0.05$). **c)** In the NAcc, bLRs had greater expression of proenkephalin than bHRs (* $p < 0.05$).